

A COMPARATIVE STUDY OF FACTORS WHICH INFLU-  
ENCE THE CHEMICAL COMPOSITION OF  
SOME KERATINS

By WALTER DAVID BLOCK

A DISSERTATION SUBMITTED IN  
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE  
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## THE AMINO ACID CONTENT OF COW AND CHIMPANZEE HAIR

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Interest in more exact knowledge of the chemical composition of the keratins of various types has been greatly stimulated by the results obtained by the use of the methods of x-ray analysis as a means of detecting and measuring regularities of the molecular patterns of the fibrous keratins (1). For formulation of such patterns, more complete analyses of the amino acid components of the keratins are essential. The most complete and more recent studies have been concerned with wool, the chief fibrous keratin of commercial importance.

Further interest in keratins has been aroused by the recently proposed characterization of a true keratin (2, 3) as a protein with a relatively constant molecular ratio (1:4:12) of the basic component amino acids, histidine, lysine, and arginine. The constancy of this ratio suggests the presence of some uniform nucleus in the molecules of keratins derived from various biological sources.

Another characteristic of the fibrous keratins is their high content of sulfur and the sulfur-containing amino acid, cystine. Keratinization of a fiber has been associated with the process of cystinization (4, 5). Factors, age and diet, which may influence the hair of young white rats, have been investigated previously in this laboratory (6, 7). Workers in the Japanese laboratories have also studied variations of the cystine content of hair, related to species, sex, and age (8, 9).

No recent study of cow hair was available and we had been much interested in the report by Hsieh (10), that only 1.5 to 2.5 per cent of cystine could be isolated from cow hair. Since 5 to 6 per

cent of cystine can be obtained from human hair in isolations in which no attempt at quantitative procedures is made, the findings of Hsieh suggested a content of cystine in cow hair much lower than that of other animal fibers. The analyses of Okuda and Katai (9) were not yet available when this study was undertaken. Through the helpful cooperation of Professor G. L. Huffman of the Department of Dairy Husbandry of Michigan State College, we were able to obtain for study samples of hair from cows of pure breed (Holstein), and of known age and nutritive history. It was thus possible to secure more complete data concerning the amino acids of cow hair and also to study the age factor in cows of the same breed and herd in relation to the cystine content of the hair.

It has been possible to study the composition of the hair of chimpanzees of known sex and age with especial reference to the content of cystine.

#### EXPERIMENTAL

For extraction of the hair prior to analysis the samples were suspended in distilled water for 12 hours at room temperature, dried on filter paper in the laboratory for 12 to 20 hours, and then extracted in a continuous extraction apparatus at 45-50° with the various solvents. The extraction was carried out with 95 per cent alcohol, chloroform, absolute alcohol, and ether in succession for periods of 48, 18, 24, and 12 hours respectively. After the final extraction, the samples were dried in a vacuum oven at 60° for 24 hours and stored in a vacuum desiccator for analysis. No alteration in the character of the fiber was observed as a result of the extraction.

Moisture and ash were determined by the usual procedure, total nitrogen by the micro-Kjeldahl method of Pregl.

Total sulfur was determined by two methods, the modified Denis-Benedict procedure as used in this laboratory (11) and the Parr bomb method. In view of the difficulties in the application of the Benedict or Denis method to the analysis of methionine or of proteins containing significant amounts of methionine (12), the Parr bomb method was used in parallel determinations with the modified Denis-Benedict method. Since the keratins contain little methionine, it was believed that the use of the modified



Denis-Benedict method would introduce little error. This assumption proved to be justified by the results of the analyses. No significant differences in the content of total sulfur of the keratins, as determined by the two methods, were apparent. Comparative unselected analyses of six consecutive samples of cow hair gave 3.76 and 3.73, 3.90 and 3.87, 3.76 and 3.65, 3.81 and 3.69, 3.80 and 3.75, and 3.67 and 3.79 per cent of sulfur by the modified Denis-Benedict and the Parr bomb methods respectively. The values presented in Tables I and IV are those obtained by the use of the Parr bomb method.

Cystine was determined by the Sullivan method, as modified by Rossouw and Wilken-Jorden (13), tyrosine and tryptophane by the methods of Folin and Marenzi (14), basic amino acids by the methods of Block (15), and dicarboxylic amino acids by the methods of Jones and Moeller (16).

Proline was determined as described by Bergmann (17), and glycine by the method of Bergmann and Fox (18). Since, at the time this work was undertaken, Bergmann and Fox had not yet made available the exact details of their procedure, we made use of the slight modification described by Block (19) generously placed at our disposal by him. No correction for the solubility of the double salt of glycine with potassium trioxalatochromiate (20) was made.

### *Cow Hair*

The total nitrogen content of the cow hair ranged from 14.64 to 16.00 per cent, but, in general, was of the same order of magnitude in all the samples of hair and did not appear to bear any relation to the age of the animal (Table I). Total sulfur content varied only slightly in the thirteen samples analyzed (range, 3.62 to 3.87 per cent) and could not be correlated with age (Table I).

Humin nitrogen varied from 1.3 to 6.7 per cent of the total nitrogen, the higher values being obtained in the analyses of the black hair. Amide nitrogen comprised from 9.1 to 16.6 per cent of the total nitrogen. Although there appeared to be a tendency for a lower amide nitrogen in the hair of the younger group of animals (range, 9.1 to 12.1 per cent) than in the hair of the older animals (range, 13.4 to 16.6 per cent), no attempt was made to correlate these values with the content of the dicarboxylic amino

acids, since the methods for the determination of this latter group are not entirely satisfactory, and since the amount of the individual samples available for analysis was limited.

The contents of tryptophane and of tyrosine of the hair were relatively constant and also bore no relation to the age of the animal (Table I). In the case of cystine, an entirely different picture was presented. The values ranged from 10.97 to 13.58 per cent. In the group of animals 3 to 4 months of age there was

TABLE I

*Analysis of Samples of Cow Hair (Holstein) from Animals of Different Ages*

Two separate samples of hair from Animals 188 and 190 were analyzed, the one consisting of white and the second consisting of black hair, as indicated in the table. All values are calculated on an ash-free, moisture-free basis. The letters W., B., and M. indicate white, black, and mixed (black and white) hair respectively.

| Animal No. | Color | Age         | Total N         | Total S         | Cystine         | Cystine S of total S | Tyrosine        | Tryptophane     |
|------------|-------|-------------|-----------------|-----------------|-----------------|----------------------|-----------------|-----------------|
|            |       | <i>mos.</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>      | <i>per cent</i> | <i>per cent</i> |
| 193        | W.    | 3           | 15.32           | 3.73            | 10.97           | 79.2                 | 3.33            | 1.33            |
| 191        | "     | 3           | 14.64           | 3.87            | 11.45           | 78.5                 | 3.13            | 1.40            |
| 190        | "     | 3           | 15.20           | 3.65            | 11.46           | 83.8                 | 3.51            | 1.47            |
| 190        | B.    | 3           | 15.54           | 3.69            | 11.24           | 81.5                 | 3.23            | 1.43            |
| 188        | W.    | 4           | 15.65           | 3.75            | 11.46           | 81.6                 | 3.29            | 1.36            |
| 188        | B.    | 4           | 16.00           | 3.79            | 11.45           | 81.0                 | 3.25            | 1.37            |
| 189        | M.    | 4           | 15.58           | 3.63            | 11.65           | 85.8                 | 3.30            | 1.30            |
| 15         | B.    | 49          | 15.61           | 3.77            | 12.65           | 89.8                 | 3.45            | 1.42            |
| 8          | W.    | 61          | 15.51           | 3.62            | 13.42           | 98.8                 | 3.19            | 1.40            |
| 5          | "     | 61          | 15.12           | 3.75            | 13.58           | 96.6                 | 3.21            | 1.28            |
| 6          | "     | 83          | 15.55           | 3.77            | 13.26           | 93.5                 | 3.13            | 1.33            |
| 3          | "     | 86          | 15.67           | 3.66            | 13.43           | 98.2                 | 3.41            | 1.42            |
| 4          | B.    | 87          | 14.84           | 3.62            | 13.36           | 98.6                 | 3.35            | 1.29            |

a relatively small variation in the cystine content (10.97 to 11.65 per cent). In the older group, 49 to 87 months of age, the cystine content was consistently higher (12.65 to 13.58 per cent), as shown in Table I. The values for the hair of the adult animals were definitely higher than those reported by Okuda and Katai (9) and the variation with age in our own analyses is much more marked. The hair of animals younger than 24 months was not examined by them. The results obtained furnish further proof



that in the production of adult hair keratinization is accompanied by cystinization (4-6). The values for the cystine content of cow hair are comparable to those for other fibrous keratins and demonstrate clearly that there is not the low content of cystine in cow hair suggested by the isolation studies of Hsieh (10).

The cystine content was consistently higher in the hair of the older group, but the total sulfur was relatively uniform and bore no relation to the age of the animal. In the younger group, cystine sulfur comprised from 79.2 to 85.8 per cent of the total sulfur (Table I), while, in the older group, total sulfur was almost completely accounted for as cystine sulfur in most cases. While we realize the limitations of cystine determinations, we believe that the differences in the percentage of the total sulfur present as cystine sulfur are clearly beyond the experimental error of the analytical procedures and suggest the presence, in the hair of young animals, of considerable amounts of sulfur in some compound other than cystine. Further evidence in support of this hypothesis is afforded by consideration of the values for labile sulfur obtained by the method of Zahnd and Clarke (21). Since the source of labile sulfur is believed to be cystine, variations in the cystine content of the keratins should be reflected in the analyses for labile sulfur. The amount of material available limited our determinations to the analyses of four samples, two in each age group. The white hair of Animal 191 and black hair of Animal 190 yielded 2.84 and 2.80 per cent respectively of their sulfur as labile sulfur, equivalent to 72.8 and 73.5 per cent of the total sulfur. The two samples of white hair of the older group, Animals 6 and 3, gave on analysis values of 3.29 and 3.39 per cent of labile sulfur or 86.8 and 91.2 per cent respectively of the total sulfur.

Basic amino acids were determined in three samples of hair, one from the younger age group and the others from the older group (Table II). The values obtained were practically identical in all three samples and are similar to those reported previously for other animal fibers (2, 3, 22). When the percentages of these basic amino acids are expressed as a molecular ratio, the result, 1:4:10, is comparable to the ratio, 1:4:12, proposed by Block and Vickery (2, 3) in their characterization of keratins.

The amount of material at our disposal did not permit analyses

of the individual samples of hair for their content of glutamic and aspartic acid. Two samples of mixed hair, one from the younger group (Animals 190, 193, and 191) and one from the older group (Animals 3, 4, and 5) were analyzed. Values of 2.7 and 3.0 per cent of aspartic acid were obtained for the mixed samples of the hair of the younger and older groups respectively and values of 12.1 and 11.4 per cent for glutamic acid. Hair from Animals 3 and 8 contained 10.56 and 10.02 per cent of glycine respectively, and hair from Animals 4, 8, and 189, 8.28, 8.18, and 8.22 per cent of proline respectively. The average percentages of glycine and proline in cow hair were 10.28 and 8.28 per cent. The relatively high content of glycine is similar to the high figure of 9.12 per cent reported by Buchtala for human hair (23).

TABLE II  
*Basic Amino Acids of Cow Hair*

All values are calculated on an ash-free, moisture-free basis. The letters W. and B. indicate white and black hair respectively.

| Animal No. | Color | Age         | Histidine       | Lysine          | Arginine        | Molecular ratio, histidine to lysine to arginine |
|------------|-------|-------------|-----------------|-----------------|-----------------|--------------------------------------------------|
|            |       | <i>mos.</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |                                                  |
| 193        | W.    | 3           | 0.69            | 2.14            | 7.42            | 1:4:10                                           |
| 15         | B.    | 49          | 0.67            | 1.87*           | 7.48            | 1:3:10                                           |
| 4          | W.    | 87          | 0.68            | 2.10            | 7.44            | 1:4:10                                           |

\* Slight loss.

In Table III are assembled the averages of all the amino acid determinations of cow hair made in the present series and, for comparison, typical values for wool as presented by Astbury and Woods (24).

#### *Chimpanzee Hair*

Our interest in the composition of chimpanzee hair was aroused by the known evolutionary relationships between the anthropoid apes and man. There is a closer resemblance in respect to certain metabolic processes between the anthropoid apes and man than between these apes and the lower mammals. Human hair is greater in its content of cystine than is any other similar animal fiber. Is the content of cystine of the hair of the chimpanzee

similarly high? Through the courtesy of Professor R. M. Yerkes of the Laboratory of Primate Biology of Yale University, we have secured specimens of hair from chimpanzees of known age and sex, from animals which have been under observation for prolonged periods of time in the colony at Orange Park in northern Florida (26). For comparison we present analyses of three samples of human hair also (Table IV).

TABLE III

*Amino Acid Content of Cow and Chimpanzee Hair and Sheep Wool*

The values for wool are those compiled by Astbury and Woods (24), except where noted.

| Amino acid         | Cow hair        | Wool            | Chimpanzee hair |
|--------------------|-----------------|-----------------|-----------------|
|                    | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Histidine.....     | 0.67            | 0.6             | 0.64            |
| Tryptophane.....   | 1.37            | 1.8             | 1.42            |
| Lysine.....        | 2.03            | 2.8             | 2.02            |
| Aspartic acid..... | 2.86            | 7.3*            |                 |
| Tyrosine.....      | 3.29            | 4.8             | 3.32            |
| Arginine.....      | 7.45            | 10.2            | 8.09            |
| Proline.....       | 8.28            | 4.4             |                 |
| Glycine.....       | 10.28†          | 0.6             |                 |
| Glutamic acid..... | 11.76           | 15.3*           |                 |
| Cystine.....       | 13.41‡          | 13.1            | 15.50§          |

\* Values obtained by Speakman and Townsend (25).

† If the glycine value is corrected (20), a figure of 11.68 per cent is obtained.

‡ The values for cystine are averages of the analyses of the hair from the five animals whose age is greater than 60 months. The values for these animals are included because of the greater uniformity of the cystine content of the hair of the older animals.

§ Average of analyses of hair from three adult animals only.

The hair of the chimpanzee (Table IV) contained a higher percentage of sulfur and of cystine than cow hair, a percentage of sulfur only slightly less than that of the samples of human hair analyzed in the present study. There was observed a tendency toward a greater percentage of cystine of the hair of the older animals. Thus, the average cystine value for the hair of the two younger animals was 14.55 per cent and that of the samples of adult chimpanzee hair, 15.50 per cent. The percentage of cystine



in chimpanzee hair was similar to that in the three samples of human hair analyzed. The content of total sulfur did not vary but, as in the studies of cow hair, it was possible to account for a greater percentage of the total sulfur of the hair as cystine sulfur in the older animals (Table IV).

Tyrosine and tryptophane values were relatively uniform for all the samples of chimpanzee hair and were comparable to those already presented in our analyses of cow hair. It was possible to determine the basic amino acids in a single sample of chimpanzee hair only (Animal 4). The percentages of histidine, lysine, and

TABLE IV

*Chemical Composition of Hair of Chimpanzee and of Man*

All values are calculated on an ash-free, moisture-free basis.

|            | Animal No. | Sex  | Age  | Total N  | Total S  | Cystine  | Cys-tine S of total S | Tyro-sine | Tryp-to-phan |
|------------|------------|------|------|----------|----------|----------|-----------------------|-----------|--------------|
|            |            |      | yrs. | per cent | per cent | per cent | per cent              | per cent  | per cent     |
| Chimpanzee | 1          | M.   | 1.5  | 16.54    | 4.37     | 14.42    | 88.0                  | 3.16      | 1.44         |
|            | 2          | F.   | 3    | 16.62    | 4.25     | 14.67    | 92.1                  | 3.16      | 1.41         |
|            | 3          | "    | 11   | 16.66    | 4.42     | 15.70    | 95.0                  | 3.34      | 1.36         |
|            | 4          | M.   | 15   | 16.86    | 4.20     | 15.45    | 98.1                  | 3.63      | 1.44         |
|            | 5          | F.   | 17   | 16.75    | 4.30     | 15.30    | 94.9                  | 3.34      | 1.43         |
| Human      | 6          | " *  | 24   | 16.66    | 4.80     | 15.40    | 85.5                  | 2.57      | 1.42         |
|            | 7          | M. † | 24   | 16.57    | 4.83     | 15.45    | 85.6                  | 3.31      | 1.27         |
|            | 8          | " ‡  | 70   | 15.52    | 5.08     | 15.93    | 83.6                  |           |              |

\* Blond hair.

† Red hair.

‡ Gray hair.

arginine recorded in the compilation of Table III are similar to those of cow hair. The molecular ratio of the basic amino acids is approximately 1:4:10; *i.e.*, chimpanzee hair is a keratin as defined by Block and Vickery (2).

Okuda and Katai (9) have reported analyses of the cystine content of the hair of various species of monkeys, but analyzed only one sample of hair from each species. No details as to sex or age are available. The cystine values (determined by the iodometric method of Okuda) are very variable, ranging from 10.18 (gibbon) to 14.48 (pig-tailed monkey) per cent. The values ob-

tained by us in our analyses of chimpanzee hair are uniformly high and suggest that the high content of cystine of the hair is a further point of similarity between man and the anthropoid apes.

#### SUMMARY

1. In order to afford more complete data concerning the composition of a fibrous keratin other than wool, a study of the composition of cow hair, which included analyses for ten of the amino acids, has been made.

2. The variations in composition associated with the age of the animal have been studied by the examination of thirteen samples of hair from pure bred Holstein cows of the same nutritive history, but varying in age from 3 to 87 months. The cystine content ranged from 10.97 to 11.65 per cent in the hair of younger animals (3 to 4 months) and from 13.26 to 13.58 per cent in the hair of adult cows (61 to 87 months).

3. The hair of the chimpanzee resembled human hair in the high content of total sulfur and cystine. The cystine content of the hair of older animals was slightly higher than that of the younger animals, but the total sulfur did not vary.

4. The molecular ratios of the three basic amino acids of both cow and chimpanzee hair were such as to characterize these fibrous proteins as keratins, as defined by Block and Vickery (2).

#### BIBLIOGRAPHY

1. Astbury, W. T., *Compt.-rend. trav. Lab. Carlsberg*, **22**, 45 (1938).
2. Block, R. J., and Vickery, H. B., *J. Biol. Chem.*, **93**, 113 (1931).
3. Block, R. J., *J. Biol. Chem.*, **121**, 761 (1937).
4. Bekker, J. G., and King, A. T., *Biochem. J.*, **25**, 1077 (1931).
5. Jordan-Lloyd, D., and Marriott, R. H., *Biochem. J.*, **27**, 911 (1933).
6. Lightbody, H. D., and Lewis, H. B., *J. Biol. Chem.*, **82**, 485, 663 (1929).
7. Heard, E. V., and Lewis, H. B., *J. Biol. Chem.*, **123**, 203 (1938).
8. Tadakoro, T., and Ugami, J. H., *J. Biochem., Japan*, **12**, 187 (1930); **15**, 25 (1932).
9. Okuda, Y., and Katai, K., *J. Biochem., Japan*, **24**, 207 (1936).
10. Hsieh, Y., *Science, China*, **15**, 891 (1931).
11. Wilson, R. H., and Lewis, H. B., *J. Biol. Chem.*, **73**, 543 (1927).
12. Painter, E. P., and Franke, K. W., *J. Biol. Chem.*, **114**, 235 (1936).
13. Rossouw, S. D., and Wilken-Jorden, T., *Onderstepoort J. Vet. Sc. and Animal Ind.*, **2**, 361 (1934).
14. Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, **83**, 89 (1929).

15. Block, R. J., *J. Biol. Chem.*, **106**, 457 (1934).
16. Jones, D. B., and Moeller, O., *J. Biol. Chem.*, **79**, 429 (1928).
17. Bergmann, M., *J. Biol. Chem.*, **110**, 471 (1935).
18. Bergmann, M., and Fox, S. W., *J. Biol. Chem.*, **109**, 317 (1935).
19. Block, R. J., The determination of the amino acids, Minneapolis, 64 (1938).
20. Bergmann, M., and Niemann, C., *J. Biol. Chem.*, **122**, 577 (1937-38).
21. Zahnd, H., and Clarke, H. T., *J. Biol. Chem.*, **102**, 171 (1933).
22. Stewart, A. M., and Rimington, C., *Biochem. J.*, **25**, 2189 (1931).
23. Buchtala, H., *Z. physiol. Chem.*, **85**, 246 (1913).
24. Astbury, W. T., and Woods, H. J., *Tr. Roy. Soc. London, Series A*, **232**, 333 (1933).
25. Speakman, J. B., and Townend, F., *Nature*, **139**, 411 (1937).
26. Yerkes, R. M., *Harvey Lectures*, **31**, 57 (1935-36).





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